**SYNOPSIS**

For

**The Ph.D. Research Work of Ranchi University**

Titled

# EFFECT OF CATECHIN-COATED DIALDEHYDE CELLULOSE ON THE TREATMENT OF ATHEROSCLEROTIC LESIONS IN ALBINO RAT.

To be undertaken by

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**TITLE**

**Effect of catechin-coated dialdehyde cellulose on the treatment of atherosclerotic lesions in the albino rat.**

## STATE OF THE ART

With the advancement in technology, life has become more sedentary. There is a lack of physical activity which causes obesity and high cholesterol levels. (Perrone MA,2021) Consumption of processed food, alcohol, and cigarette smoking has increased, affecting the heart muscles, and blood vessels. (Mukamal KJ, 2006) As a result, cardiovascular disease has become one of the primary causes of mortality worldwide posing great threats to public health. Due to an increase in the amount of trans fat in the diet, the level of LDL increases in the body. (NIH,2022) The presence of Reactive Oxygen Species (ROS) causes the oxidation of LDL which in turn causes the LDL to become more susceptible to macrophages. (Khatana C, 2020). The oxidized LDL is taken inside the macrophage through a Scavenger receptor-mediated pathway. The accumulation of oxidized LDL in excess within macrophages leads to the formation of foam cells which is the major cause of atherosclerosis. (Zhao, J. F, 2012). An inflammatory response is generated by the foam cells and influences other immune cells to be attracted to the site of inflammation. Ultimately, a fatty streak is formed which progresses into a fibrous plaque within the arterial wall leading to atherosclerosis. (Guerrini, V., 2019).

Many drugs are available in the market that promise to lower cholesterol levels. They proved effective in lowering the LDL level but were ineffective in the degeneration of already-formed plaque. (Okuyama, H, 2007). Statin is a widely used drug for cardiac diseases however, in recent studies, it has been found that statin is toxic to mitochondria, inhibits the synthesis of vitamin $K_2$, accelerates calcification of the arteries, and increases the risk of heart failure. (Okuyama, H, 2015). Thus, there is a need for new antioxidative strategies to prevent the oxidation of LDL and oxidative stress. (Poljsak, B, 2011)

Catechin, a green tea polyphenol, is the most abundant flavonoid in green tea extracts. It provides a variety of vascular protective actions, including antioxidative, antihypertensive, anti-inflammatory, anti-proliferative, anti-thrombogenic, and lipid-lowering properties. (Anandh Babu, 2008). Studies have found that green tea consumption is associated with a reduced risk of cardiovascular disease. (Gao N, 2022). Catechin has shown potential in the prevention and treatment of a variety of ailments like decreasing blood pressure, inhibiting oxidative damage, and reducing lipid peroxidation in vascular smooth muscles. (Chako, S.M, 2010). Although catechin has excellent medicinal benefits, it has several downsides, such as instability in dilute solutions, lack of target specificity, and bioavailability. To overcome its low stability, catechin has been effectively conjugated to a variety of polymers including chitosan, and starch, resulting in increased stability and antioxidant activity. (Oliver, S, 2016).

As the phenolic polysaccharide conjugate has greater potential for antioxidant activity, hence can be investigated as a new antioxidant. (Liu, J, 2017). This suggests that phenolic polysaccharide conjugates can be investigated as new antioxidants. (Vittorioa, O, 2012). So far, phenolic compounds have been effectively grafted onto many types of polysaccharides, including chitosan, dextran, inulin, and starch. Other phenolic-polysaccharide conjugates

were rarely reported. (Liu, J, 2018). We are focusing on cellulose-dialdehyde polymers coated with catechin as a treatment for atherosclerosis as the catechin polymers are established as non-toxic nanoparticles for drug delivery.

Sodium periodate-mediated cellulose oxidation selectively oxidizes the vicinal hydroxyl groups in the C2 and C3 positions to aldehydes, resulting in the breaking of the C2−C3 link. It leads to the formation of 2,3-dialdehyde groups without significant side reactions. This process is one of the most effective for cellulose modification. (Lindh, J, 2014).

Natural polysaccharides are important biopolymers that possess beneficial biological properties. (Liu, J, 2020). However, natural polysaccharides have extremely limited antioxidant activity because they lack adequate hydrogen atom- and/or electron-donating groups. (Wang, J, 2016). Conjugation of polyphenols onto polysaccharides can boost antioxidant activity. (Hu, Q, 2016). Furthermore, the synthetic polysaccharide-polyphenol conjugates are often non-toxic and can be employed in active packaging, edible coating, encapsulation, transport, and bio-adsorption applications. (Liu, J, 2018). Existing research has shown that conjugating catechin onto various polysaccharides can improve catechin stability while also increasing polysaccharide antioxidant activity. (Hu, H, 2021). Till now, different kinds of polyphenols have been successfully conjugated with Dialdehyde cellulose. (Cirillo, G, 2012) but very little work has been done on the conjugation of catechin with dialdehyde cellulose and hence this study is been undertaken to analyze the efficacy of catechin-coated DAC as a reliable treatment of atherosclerosis.

## PROBLEMS TO BE INVESTIGATED

- To use catechin-DAC conjugate as a polymer for drug delivery.
- To investigate the efficacy of catechin-DAC conjugate as a potential antioxidant for the treatment of atherosclerosis.
- To provide a natural alternative for the treatment of atherosclerosis and solve the issues faced by using drugs.

## OBJECTIVES OF THE INVESTIGATION

1. To form DAC with a catechin coat.
2. To study the characteristics of DAC-Catechin using TEM, FT-IR, and UV-Vis.
3. To create an atherogenic rat by making dietary modifications.
4. To administer DAC-catechin in rats intravenously to study the antioxidative effects.
5. To determine the total aortic cholesterol in rats.

## AIM OF THE STUDY

- Contribute to the creation of a natural technique for treating atherosclerosis and solving the issues faced by using drugs.
- To provide valuable insights into the potential of the application of catechin-coated DAC in the treatment of atherosclerosis, promoting the use of natural alternatives to synthetic drugs.

## HYPOTHESIS

It is expected that the synthesized catechin-DAC conjugate will be effective in the treatment of atherosclerosis.

## RESEARCH DESIGN

The research design involves the preparation of catechin-conjugated DAC and checking the antioxidant effect of catechin. It also involves intravenous injection of catechin-DAC into rats, and studying the cholesterol levels in the aorta. All the groups are monitored, including weight, HDL, LDL, and plaque formation. Data on these changes are collected and statistically analyzed to compare the groups.

## METHODOLOGY

### Extraction of catechin from green tea leaves

Green tea is obtained from the market. The leaves are dried in a hot air oven, crushed to powder form, and brewed in a reflux apparatus at 60 degrees Celsius for 10 minutes. (Bharadwaz, A, 2012) The hot water extraction technique is used for the extraction of catechin (Vuong, QV, 2010). The extract is filtered and partitioned with water: chloroform for decaffeination. A second partition is done using water: ethyl acetate to obtain catechin and remove any other substances (Row, KH, 2006). The extracted catechin is detected via HPLC (Scoparo, CT, 2012).

### Synthesis of DAC

In brief, 500 mL of deionized water was used to dissolve 13.2 g of sodium periodate, and the resultant solution was adjusted with hydrochloric acid to pH 2.0. Microcrystalline cellulose powder (10gm) was mixed with $NaIO_4$ and stirred continuously. The reaction was carried out in the dark at 30 degrees Celsius for 21 hours. Then, 35ml of ethylene glycol was added to remove the unreacted periodate. The solution was centrifuged at 5000rpm for 15 minutes, and the solid was washed with distilled water repeatedly. The product was dried at 35 degrees Celsius for 6 hours and crushed. DAC was obtained as a white solid. (Zhang, L., 2017).

### Synthesis of DAC-Catechin conjugate

Both DAC and Catechin were dissolved in deionized water respectively. 0.125g of DAC was dissolved in 3.3 ml of 1mol/L of hydrochloric acid. Different amounts of catechin (0.063, 0.125, and 0.188 g) were dissolved in 6.7 mL of DMSO. (Hu, H, 2021) Both were mixed and reacted in the dark at 40 degrees Celsius for 48 hours. The solution was dialyzed against distilled water and lyophilized. (Oliver, S, 2016).

### Preparation of atherogenic rat

The rats were tested for blood cholesterol levels by performing lipid profiles before they were fed. After it was confirmed that the blood cholesterol levels were normal, rats were separated into control and test groups. The control group is fed with commercially available rat food and the test group is fed with the rat food along with 10% linoleic acid, 1.5% cholesterol,

0.5% cholic acid, and 51.2% sucrose. Each mouse was given a diet (6 g/day) for 14 weeks. By monitoring the aortic cholesterol ester levels, we can know whether the rat developed atherosclerotic lesions or not. (Yu, Y., Satomi, 1993)

**Administration of DAC-Catechin into rat**

Rat are injected intravenously with DAC-Catechin solution. They were injected twice a week for three weeks.

**Determination of aortic cholesterol**

The aorta is removed from the heart. The adipose tissue is separated from it. The aorta is then stained with Oil Red O for 30 min and then washed off with 60% isopropanol. (Wan, J, 2023)

**Statistical Analysis**

The obtained result can be analyzed using a one-way ANOVA test if the experiments consist of more than two groups, otherwise, an unpaired t-test can be performed.

## REQUIREMENTS

Chemicals- Chloroform, ethyl acetate, microcrystalline cellulose, sodium meta periodate, ethylene glycol, distilled water, deionized water, ferric chloride, formalin, isopropanol, oil red o stain, coconut oil, rat food pellet

Instruments- HPLC, Reflux apparatus, beaker, funnel, conical flask, filter paper, hot air oven, measuring cylinder, centrifuge, centrifuge tubes, spatula, micropipette, pp bottle

## SIGNIFICANCE OF THE STUDY

This study will hold significant importance for the medical industry. The research will offer a potential solution for treating atherosclerosis by investigating the use of catechin-DAC. It will also ensure safer alternatives for patients as compared to synthetic drugs. Additionally, the study will contribute to the field of health by highlighting the advantages of natural medicines over synthetic alternatives while also exploring the application of nanotechnology in the health industry. The findings will have practical implications for the treatment of atherosclerosis and advance the knowledge of nanotechnology in the medicinal field.

## RELEVANCE TO THE PRESENT-DAY PROBLEM AND NEED OF THE SOCIETY

This study is highly relevant to the population's increasing heart disease problems. It addresses the demand for natural and safe alternatives that can minimize the side effects caused by synthetic products. It also aligns with the societal need for better and natural alternatives instead of loading the body with steroids which is very harmful in the long run.

## CONTRIBUTION TO EXISTING KNOWLEDGE

Nanotechnology is an advancing industry and extensive research is being done on using green synthesized nanoparticles as a natural treatment. Many plant-derived nanoparticles have been studied and their antioxidative and other properties have been investigated in

detail. However, very little research has been conducted using catechin as an antioxidant agent in rats. This research holds the promising ability to enhance the possibility of an alternative for the treatment of atherosclerosis.

**FUTURE WORK**

In the future, the researchers can explore the potential of other antioxidant agents found in green tea or any other plant as a remedy for the treatment of atherosclerosis, broadening the scope of the medical industry. Additionally, research can focus on evaluating the long-term impact of the DAC on the body or organs. By delving deeper into these areas, future research can enhance our understanding and utilization of natural alternatives for the treatment of various other diseases.

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